PROJECT NUMBER: 6906
PROJECT TITLE: Biological Effects of Smoke
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I. PROTEIN KINASE C (PKC) WHOLE CELL ASSAY: EFFECTS OF TPA and CSC

A. Objective: Evaluate the effects of 12-O-tetradecanoyl-phorbol-13-acetate (TPA) on protein phosphorylation in quiescent 3T3 cells.

B. Results: TPA increased protein phosphorylation in cells grown in both standard and high glucose media. However, the cells grown in high glucose media exhibited a greater response than cells grown in standard media. A 5 hr incubation with IT 2R1 CSC did not affect protein phosphorylation relative to the solvent control in high glucose 3T3 cells. Results obtained with the standard media were inconclusive.

C. Plans: Expand the evaluation of IT 2R1 CSC on cells grown in standard media and high glucose media to include additional incubation times.

D. References: Nixon, G. , Notebook No. 8569, p. 146.

II. INHIBITION OF EGF BINDING ASSAY: TIME COURSE

A. Objective: Determine how the effects of CSC and catechol on EGF binding and cell numbers change with time.

B. Results: A second time course experiment examining the 17.5 to 22.5 hr time-span confirmed the results of the first experiment. The effects of IT 2R1 CSC, IT 100% ES CSC and catechol on EGF binding and cell numbers did not significantly change over this time period. Two experiments were conducted which evaluated treatment times from 0 to 7 hr. The inhibition of EGF binding caused by IT 2R1 CSC, IT 100% ES CSC and catechol increased rapidly with time up to 7 hr. However, there were no changes in cell numbers by 7 hr. The effects of IT 2R1 CSC and IT 100% ES CSC were the same throughout the time periods examined. The inhibition of EGF binding and reduction in cell numbers by catechol and CSC followed the same time course.

C. Plans: Continue to use the standard 19.5 hr treatment time in future experiments.

D. References: Patskan, G. Notebook No. 8664, p. 118.

III. PDBu BINDING ASSAY

A. Objective: Determine the effects of catechol and benzo(a)pyrene (B(a)P) on PDBu binding to 3T3 cells.

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B. **Results**: Experiments confirmed previous findings that catechol increased PDBu binding after a 19.5 hr treatment period but had no effect when added at the time of binding. B(a)P produced a weak stimulation of PDBu binding following a 19.5 hr treatment and no effect when administered at the time of binding.

C. **Plans**: Examine the effects of protein kinase C inhibitors on the increase in PDBu binding elicited by CSC.

D. **References**: Ferguson, T.J. Notebook No. 8645

IV. GLUTATHIONE DEPLETION ASSAY (GDA):QUANTITATION of the GLUTATHIONE (GSH) LEVEL in SALMONELLA STRAIN TA98

A. **Objective**: To determine the GSH level in an overnight culture of Salmonella strain TA98.

B. **Results**: Using a modified version of the procedure for GSH extraction currently used in the standard GDA, HPLC analysis indicated the presence of 265 nmol GSH/$6X10^{10}$ TA98 cells. These results compared well to 146 nmol/$6X10^{10}$ cells reported in the literature. The GSSG level at the same cell concentration was found to be 21 nmol vs 9 nmol reported in the literature.

C. **Plans**: To determine at what level 2R1 CSC depletes GSH in strain TA98.

D. **References**: McCoy, W. R. Notebook No. 8555, pp.118-121.

V. GDA: EFFECT OF 2R1 CSC ON THE GSH LEVEL in SALMONELLA

A. **Objective**: To determine the effect of 2R1 CSC on the GSH level in Salmonella strain TA98 as they are used in the Salmonella assay.

B. **Results**: TA98 cells were treated with 2R1 CSC for 0, 1 and 2 hr (37°C in incubator) using an assay protocol similar to that routinely used in the Salmonella assay. The results showed a significant reduction in GSH compared to the solvent (DMSO) control at 0 and 1 hr treatment times (55 and 72% respectively). However, after 2 hr of treatment, GSH recovered to control levels.

C. **Plans**: Determine the effect on the S/M activity of 2-nitrofluorene using TA98 cells which have been GSH depleted by 2R1 CSC.

D. **References**: McCoy, W.R. Notebook No. 8555, pp.118-121.

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VI. SALMONELLA/MICROSOME (S/M) ASSAY -- ADDITION OF d-ALANINE TO 1R4F CONTROL FILLER

A. **Objective**: This experiment was designed to determine if the addition of d-alanine to 1R4F filler would alter the S/M IT CSC activity as compared to the solvent control.

B. **Results**: D-alanine was dissolved in saline and oversprayed onto 1R4F filler at three levels (0.5, 1.0 and 2.0%). The data indicated that 1R4F + solvent control was significantly higher in IT CSC specific activity (S.A.) than that of 1R4F alone. Increasing the concentration of d-alanine in 1R4F increased the IT CSC S.A.s compared to the solvent control. However, there was no significant difference between CSCs from 1R4F filler containing 0.5 and 1.0% added d-alanine.

C. **Plans**: To evaluate the effects of adding various nitrogenous tobacco components to samples in the crossed soluble/baseweb study.

D. **Reference**: Thompson, L. H. Notebook No. 8628, pp. 110-119.

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